Akciğer Tüberkülozlu Olgularda Balgam Mikroskopi - Kültür Pozitifliğini Etkileyen Faktörler: Tanı İçin Kaç Mikroskopi?

Factors Influencing Sputum Smear and Culture Positivity in the Diagnosis of Pulmonary Tuberculosis: How Many Specimens do We Need for Diagnosis?

Akciğer Tüberkülozu: Tanı İçin Kaç Mikroskopi?
Pulmonary Tuberculosis: How Many Specimens Need For Diagnosis?

Hüseyin Lakadamyalı[1], Hatice Lakadamyalı[2], Tarkan Ergün[2], Seref Ozkara[3]
[1]Department of Pulmonary Diseases, Baskent University, Alanya Teaching and Medical Research Center, Alanya,
[2]Department of Radiology, Baskent University, Alanya Teaching and Medical Research Center, Alanya,
[3]Department of Chest Diseases, Atatürk Chest Diseases and Chest Surgery Educational and Training Hospital, Ankara, Turkey.

Özet

Amaç

Bu çalışmada amaçlanan, TB tanısı konan olguların mikrobiyolojik verilerini ortaya koyarak, tüberküloz tanısında kullanılacak örnek sayısını belirlemek, radyolojik değişiklikler, yaş ve ek hastalıkların mikrobiyolojik sonuçlarla olan ilişkisinin araştırılmasıdır.

Gereç ve Yöntemler

Çalışmaya toplam 140 erkek hasta alınmıştır. Hastaların tamamından antitüberküloz tedavi başlanmadan önce en az üç adet mikroskopi ve kültür öreneği ile birlikte posterioanterior ve lateral göğüs grafileri çekildi. Mikroskopik değerlendirme için Ehrlich-Zeihl-Neelsen (EZN) yöntemi ve kültür ortamı olarak sıvı (BACTEC) ve/veya Löwenstein-Jensen katı besiyeri kullanıldı.

Bulgular

Balgam örneklerinin tüberküloz tanısına sağladığı katkıya bakılacak olursa, ilk mikroskopi pozitifliğinin %71.2, ikinci mikroskopi pozitifliğinin %17.6 ve üçüncü mikroskopi pozitifliğinin katkısının %7.2 düzeyinde olduğu (ilk üç mikroskopinin katkısını toplamda %96) anlaşılmaktadır. Kültürde üreme saptama oranının ise %67.14'tür. İlk üç kültürde saptanan kültür pozitifliği ise %93.61 olarak hesaplanmıştır. Olguların %21.5'de (30/140) ek hastalığı olduğu anlaşılmıştır.

Sonuç

Akciğer tüberkülozu tanısı almış hastaların mikrobiyolojik verilerine bakıldığında, hastalığın tanısı için üç balgam örneği alınması yeterli gözükmektedir. Göğüs grafisinde kavite varlığı mikroskopi pozitifliği görülme oranını arttırmaktadır. Ayrıca ek hastalığın varlığı ile kavite oluşumunu ve mikroskopi pozitifliği arasında da pozitif bir ilişki vardır. Uzamış semptom süresi ile kavite oluşumu ve ilk mikroskopi pozitifliği oranları belirgin korelasyon göstermektedir.

Anahtar Kelimeler

Akciğer Tüberkülozu, Balgam Mikroskopisi, Kültür, Tanı.

Abstract

Aim

The aim of the present study is to determine the number of specimens needed to diagnose tuberculosis basing on microbiological data and to investigate the relationship between microbiological results radiologic changes and comorbid conditions.

Material and Methods

A total of 140 male cases were included study. At least three tuberculosis microscopic exams and cultures, erect posterio-anterior and lateral chest radiographs were obtained for each patient prior to initiation of antituberculous therapy. Liquid broth (BACTEC) and/or Lowenstein-Jensen solid culture media were used to grow the microorganism and the Ehrlich-Ziehl-Neelsen (EZN) method was used for the microscopic evaluation in the study.

Results

The contribution of the first microscopic examination to the diagnosis was 71.2%, of the second microscopic examination 17.6%, and the third one 7.2% (the total contribution of the first three microscopic examinations was 96%). The distribution of cases with regard to culture positivity revealed 67.14% to have growth on culture. The ratio of culture positivity of the first three cultures was calculated as 93.61%. In 21.5% (30/140) of the cases additional medical condition was present.

Conclusions

Microbiological data of patients diagnosed with tuberculosis reveals that 3 sputum microscopies are sufficient for diagnosis. Presence of cavitary lesions on chest radiographs increases the ratio of microscopy positivity. In addition, a positive relation exists between presence of comorbidity and cavity formation and microscopy positivity. A significant correlation exists between longer symptoms' duration and cavity formation and first microscopy positivity.

Keywords

Pulmonary Tuberculosis; Sputum Smear; Culture; Diagnosis.

DOI: 10.4328/JCAM.210 Received: 19.02.2010 Accepted: 05.04.2010 Printed: 01.01.2011 J Clin Anal Med 2011;2(1):25-8
Corresponding Author: Hatice Lakadamyali, Baskent University, Alanya Teaching and Medical Research Center, Department of Radiology, 07400, Alanya, Turkey. Phone: +90-242-5112511 Fax: +90-242-5112350 E-mail: hlakadamyali@gmail.com

Introduction

Tuberculosis (TB) is a health problem which is being neglected nowadays. Presently, one third of the world population is infected with the tuberculosis bacillus. The World Health Organization's (WHO) updated incidence estimations based on surveillance and screening results are as follows: 8.8 million new cases in 2003 (140 per 100.000 population), 3.9 million of those (62 per 100.000 population) are microscopically positive and 674 000 (11 per 100.000 population) of them are estimated to be HIV infected. Prevalence figures are as follows: 15.4 million (245/100.000), 6.9 million of which are microscopically positive. 1.7 million people died of tuberculosis in 2003 (28/100.000), among those were co-infected with HIV and TB (229.000) [1].

Data from Turkey reports the following: case rate 28.5/100,000 mostly affected groups are the very young (mean age 25-34) and males (male: female = 1.9) [2].

The aim of the present study is to determine the number of specimens needed to diagnose tuberculosis basing on microbiological data of already diagnosed TB cases, and to investigate the relationship between microbiological results and age, radiologic findings and co-morbid conditions.

Material and Methods

Patients diagnosed with tuberculosis and hospitalized for treatment in our tuberculosis ward within one year period were included in the study. The laboratory records of these patients were obtained following file recording. New patients were defined as those treated for less than a month, while the rest were defined as old patients.

At least three tuberculosis microscopic exams and cultures were obtained prior to initiation of antimicrobial therapy, using sterile disposable plates. Fasting sputum collection was accomplished in the early morning, gastric irrigation (using 50cc syringe attached to a 12F nasogastric catheter the stomach of a fasting patient was irrigated and aspirated with 50cc saline) and bronchial lavage were the procedures of choice for patients incapable of producing sputum.

Liquid broth (BACTEC) and/or Lowenstein-Jensen solid culture media were used to grow the microorganism and the EZN method was used for the microscopic evaluation in the study. Microscopic examination with an oil immersion objective (x1000) was used following EZN staining and results were reported as follows: 4+ - more than 9 Acid Resistant Bacteria (ARB) per field; 3+ - 1-9 ARB per field, 2+ - 1-9 ARB per 10 fields, 1+ - 1-9 ARB per 100 fields. The "concentration method" was used to more effectively examine the sputum specimens microscopically.

Erect posterio-anterior chest radiograph and right or left (depending on lesion side) lateral erect chest radiographs were obtained for each patient prior to initiation of anti-tuberculous therapy. The radiographs were evaluated by at least one pulmonology physician and one radiology physician. In cases of scoring differences, the physicians meet together and decide on a common score. A score scale of 0-4 was used for evaluation: 0-for normal radiographs; 1-for radiographs with scattered non-cavitary lung lesions, unilaterally or bilaterally, with total lesion area not exceeding 1/5th area of one lung; 2-for radiographs with lesions of mild to moderate intensity scattered throughout one or both lungs and total area not exceeding 1/3d of total lung lesion, or total diameter of all cavitary lesions not exceeding 4cm; 3-for radiographs with lesions of mild or high intensity which have extension more than that of moderately advanced lung lesions and with above 4cm diameter of present cavities; 4 – for radiographs showing total bilateral parenchymal destruction, diffuse fibro-nodular structures and giant cavities.

Patients included into the study were classified as follows: sputum microscopy positive, sputum microscopy negative, culture positive pulmonary tuberculosis, and case definition (new case, relapse, failure of therapy, returning case that discontinued therapy for a while, chronic case).

Statistical evaluations were carried out using Statistical Package for Social Sciences (SPSS Inc. Chicago, IL, USA) 11.0 for Windows. Inter-variable comparison was done with Pearson Chi-Square test, where $p<0.05$ was deemed to have statistical significance.

Finding

A total of 140 male cases were included into the study, ages between 18 - 82 years (mean age 34.6±7.1 years). Basing on case definition, their distribution was as follows: 93 new cases, 15 relapse cases, 2 therapy failure cases, 5 returning cases, and 25 chronic cases.

The microscopic examination of the total of 525 sputum samples obtained from the cases revealed that out of 140 cases 15

Table 1. Distribution of cases based on positivity of microscopic examination

Order of smear	AFB smear + (*n*)	New case *n* (%)	Old case *n* (%)
1st	89	61 (77.21%)	28 (60.86%)
2nd	22	11 (13.92%)	11 (23.91)
3rd	9	5 (6.32%)	4 (8.69%)
4th	3	2 (2.53%)	1 (2.43%)
5th	1	0	1 (2.43%)
6th	1	0	1 (2.43%)
Total	**125**	**79**	**46**

Table 2. Distribution of cases based on positivity of culture examination

Order of Culture	Culture Positive n (%)	New Case n (%)	Old Case n (%)
1st	41 (43.61%)	30 (46.87%)	11 (36.66%)
2nd	23 (24.46%	15 (23.43%)	8 (26.66%)
3rd	24 (25.53%)	15 (23.43%)	9 (30%)
4th	4 (4.25%)	2 (3.12%)	2 (6.66%)
5th	1 (1.06%)	1 (1.56%)	0
6th	1 (1.06%)	1 (1.56%)	0
Total	94	64	30

Table 3. Distribution of cases withnregard to radiologic scoring

All Cases	Radilogic Score	New	Relapse	Chronic	Returning	Treatment Failure
2	0	2	0	0	0	0
3	1	3	0	0	0	0
5	2	5	0	0	0	0
36	3	28	5	1	1	1
92	4	53	10	24	4	1
2	Miliyer	2	0	0	0	0
140		93	15	25	5	2

had no microscopic positivity, while the ratio of cases with positive first three microscopic examinations was 96% (120/125). The contribution of the first microscopic examination to the diagnosis was 71.2%, of the second microscopic examination – 17.6%, and the third one – 7.2%. On the other hand, the total contribution of the first three microscopic examinations was 96% (Table 1).

The distribution of cases with regard to culture positivity revealed 94/140 (67.14%) to have growth on culture. The distribution of culture positivity with regard to cases revealed 64/94 (68.08%) new cases, 30/94 (31.91%) old cases. The ratio of culture positivity of the first three cultures was calculated as 88/94 (93.61%) (Table 2). The positivity ratio of the first culture was 43.61%, of the second culture – 24.46%, and of the third one – 25.53%. In 15 cases with microscopy positivity no growth was detected in culture, in 7 cases with negative microscopy the cultures were positive.

The relation between radiologic scoring and microscopy positivity was as follows: 2 of the 3 cases with radiologic score 1 the first microscopy was positive, the remaining 1 had negative microscopy and negative culture; out of the 5 cases with score 2, the first microscopy was positive in two cases, the second microscopy was positive in one case, the 6th microscopy was positive in one case, and in one case the microscopy was negative but the culture was positive, and therapy was initiated; out of the 36 cases with radiologic score 3, 32 had their first microscopy positive, 2 had the second microscopy positive, one had the 3rd microscopy positive and one had the 6th microscopy positive; out of the 92 cases with radiologic score 4, 62 had the first microscopy positive, 19 had the second microscopy positive, 6 had the 3rd microscopy positive, one had the 4th microscopy positive, and the microscopy negative cases had at least two of their cultures positive. Out of the two cases with miliary appearance one had the second microscopy positive; the other one had the 3rd microscopy positive (Table 3). All of the 80 cases with cavitary lesions radiologically had one of the six microscopies positive.

In 21.5% (30/140) of the cases additional medical condition was present. The most frequently encountered three comorbid conditions were Diabetes Mellitus (DM) 19/30 (63.33%), chronic alcoholism and malignancy, respectively. The relation between the comorbid condition (the most frequent 3 conditions were considered) and microscopy positivity revealed the following: out of the 19 cases with DM 11 had their first microscopy positive, 5 had the second microscopy positive, 3 had the 3rd microscopy positive. One of the two cases with chronic alcoholism had the first microscopy positive; the other one had the second microscopy positive. Both of the two cases with malignancy had their first microscopy positive, they were diagnosed using bronchial lavage. As a result, in 30 of the 140 cases with an existing comorbidity, the first microscopy positivity was 70 %, and the positivity of the first three microscopies was 100%.

As a result, there was a significant difference between cavity formation ($p<0.0001$) and first microscopy positivity ($p<0.0001$) between groups with comorbidity and without comorbidity.

When investigating the relation between age, radiologic score and sputum microscopy, the cases were classified into two groups: group I included 17 cases of age above 65. Only one of them had a radiologic score 2. Five of them had radiologic score 3, 11 scored 4, and 6 of them had cavitary lesions. The first sputum microscopy in 12 of the cases was positive, while negative in only 2 cases. In one case the second microscopy was positive, in one- the 3rd microscopy was positive and in the remaining one case the 4th microscopy was positive. Group II included 123 cases aged less than 65 years. Of those 64.22% (79/123) were new cases. Out of them, 79 had the first microscopy positive, 23 had the second microscopy positive, 7 had the 3rd microscopy positive, one had the 5th microscopy positive, and 3 had the 6th microscopy positive.

In 22 cases gastric lavage was used for diagnostic purposes. In 9 of those the microscopy was positive, and 12 of them had culture positivity. In two of those the microscopy was positive, while cultures being negative; and 5 were microscopy negative, while having their culture positive.

The statistical analysis revealed the following: regarding the relation between symptoms' duration and cavity formation, there was a significant difference between the group with symptoms' duration of more than 2 months, and the group with symptoms' duration 0-2 months ($p<0.0001$) (Table 4). In addition, there was a statistical significance in the ratio of first microscopy positivity ($p<0.05$) in the group with symptoms' duration of more than 2 months (Table 5). There was a significant relation between presence of cavitary lesions and first microscopy positivity and positivity of the first three microscopies ($p<0.0001$) (Table 6). Both, the presence of cavitary lesions and the microscopy positivity were found to be significantly higher in the group with comorbidity, when compared to the non-comorbidity group ($p<0.0001$).

Table 4. Relation between symptom duration and cavity formation

		Cavity			
		+	-	Total	p value
Symptom duration	0-2 month (n,%)	49 (47.6%)	54 (52.4%)	103	NS
	>2 month (n, %)	31 (83.8%)	6 (16.2%)	37	<0.0001
Total		80	60	140	

Table 5. Relation between symptom duration and first (AFB) smear positivity

		First AFB smear			
		+	-	Total	p value
Symptom duration	0-2 month (n,%)	68 (66%)	35 (34%)	103	NS
	>2 month (n, %)	31 (83.8%)	6 (16.2%)	37	0.037
Total		93	47	140	

Table 6. Relation between cavity and first (AFB) smear positivity

		First AFB smear			
		+	-	Total	p value
Cavity	+	74 (92.5%)	6 (7.5%)	80	<0.0001
	-	6 (10%)	54 (90%)	60	NS
Total		80	60	140	

Discussion

Tuberculosis continues to be a serious health problem in the developing and underdeveloped countries. Above all, the importance of early diagnosis and therapy of the disease still remains. While the culture growth of the bacterium is the gold-standard, sputum microscopy is the fastest and simplest method for its diagnosis. The diagnosis probability increases with the number of sputum microscopies. The goal, however, is to diagnose with

minimal number of sputum microscopies.

A study investigating the sensitivity of multiple sputum microscopic investigations for detecting *M. tuberculosis* in 246 cases with pulmonary tuberculosis revealed the following: in 93% (230/246) of the cases *M. tuberculosis* was detected microscopically, and in 52% of those (120/229) the number of microscopies was less than 3. The culture positivity was 25% in single culture group, 22% in the two cultures group, and 53% in the 3 or more cultures group. The effect of obtaining more than three samples was 6% on microscopy, and 0% on culture [3].

Based on our study, the contribution of multiple samples to the diagnosis of tuberculosis was estimated to be 71.2% in the first microscopy, 17.6% in the second microscopy and 7.2% in the 3rd microscopy. The contribution of more than 3 samples was noted to be limited to 4%. Regarding culture results, the first culture positivity was noted to yield 43.61%, the second culture positivity – 24.46%, and the 3rd culture positivity – 25.53% contribution. The contribution of more than 3 cultures was noted to be limited to 6.4%. While two microscopy samples seem to be sufficient, with regard to cultures - a third culture seems to be necessary for diagnosis.

Our study demonstrated that three sputum microscopies are sufficient (96%) for diagnosis, which is in concordance with literature results. The contribution of microscopies above 3, on the other hand, was quite low (4%). Sputum microscopy positivity was seen to parallel culture positivity, the ratio of bacterial growth in cultures being 93.6% for the first 3 sputum microscopy positivities.

Özkütük et al. accentuated in their study the absolute necessity of obtaining more than one microscopic samples in order to increase culture sensitivity in diagnosis of tuberculosis (4). In their opinion, two sputum samples (considering sample quality) are generally sufficient for diagnosis. However, a third sample is reported to be especially valuable for growing the bacilli in culture [4].

Longer duration of symptoms (such as cough, sputum production and weight loss) increases cavity formation paralleling microscopy positivity [5].

On radiologic images “cavity” is the most typical appearance pertaining to tuberculosis. Longer patient delay was associated with positive sputum smear and culture, and more extensive radiological disease. [5,6].

A recent study carried out in Hong Kong reported that patient delay increases microscopy and culture positivity, as well as causing diffuse radiologic disease [7].Symptoms like cough, sputum production weight loss lasting for more than 2 weeks and typical apical region infiltration on chest radiographs have a strong negative predictive value with regard of demonstration of clinical and radiological findings like cavity formation [7].

Another retrospective study showed significant relation between *M. tuberculosis* culture positivity and factors as cavitary lesions and apical region infiltration, positive tuberculin skin test, diabetes, new tuberculosis exposure and immigrant status [8].

Predictive variables include typical chest radiograph findings (cavity formation or upper zone infiltration), new exposure to tuberculosis case, positive tuberculin skin test, and lack of isoniazid prophylaxis [9].

In a prospective study designed to fast evaluation of cases presenting to hospitals with suspicion of tuberculosis demonstrated that HIV infection in addition to medical history, classical symptoms and cavitations on chest radiographs are independent predictive factors with regard to active disease [10].

Weakening of cellular and/or humoral immunity during the course of chronic illnesses (diabetes, chronic renal disease, HIV etc), and disturbed macrophage activity increase the ratio of getting tuberculosis. Thus, more attention is needed while evaluating individuals with chronic diseases. Our study demonstrated that cavity formation accompanying microscopy positivity in the group with a comorbidity were statistically significant ($p<0.0001$).

Investigating the relation between symptoms’ duration and cavity formation revealed a significant difference between the group with symptoms’ duration of more than 2 months, and the group with symptoms’ duration 0-2 months ($p<0.0001$). In addition, there was a statistical significance of first microscopy positivity in the group of longer duration of symptoms ($p<0.05$). A statistically significant relation was demonstrated between presence of cavitary lesions and first microscopy positivity and positivity of the first 3 microscopies ($p<0.0001$).

As a result, microbiological data of patients diagnosed with tuberculosis reveals that 3 sputum microscopies are sufficient for diagnosis. Presence of cavitary lesions on chest radiographs increases the ratio of microscopy positivity. In addition, a positive relation exists between presence of comorbidity and cavity formation and microscopy positivity. A significant correlation exists between longer symptoms’ duration and cavity formation and first microscopy positivity.

References

1- Global tuberculosis control: surveillance, planning, financing. WHO report 2005. Geneva. World Health Organization (WHO/HTM/TB/2005.349)

2-Handbook for Control of Tuberculosis in Turkey. 2003, Ministry of Health, Ankara

3- Nelson SM, Deike MA, Cartwrigt CP. Value of examining multiple sputum speciments in the diagnosis of pulmonary tuberculosis. J Clin Microbiol 1998; 36:467-9.

4-Özkütük A, Terek G, Çoban H, Esen N. Is it valuable to examine more then one sputum smear per patient for the diagnosis of pulmonary tuberculosis? Jpn J Infect Dis 2007;60:73-5.

5- Leung ECC, Leung CC, Tam CM. Delayed presentation and treatment of newly diagnosed pulmonary tuberculosis patients in Hong Kong. Hong Kong Med J 2007;13:221-7.

6-Leung ECC, Leung CC, Tam CM. Delayed presentation and treatment of newly diagnosed pulmonary tuberculosis patients in Hong Kong. Hong Kong Med J 2007;13:221-7.

7- Cohen R, Muzaffar S, Capellan J, Azar H, Chinikamwala M. The validity of classical symptoms and chest radiographic configuration in predicting pulmonary tuberculosis. Chest 1996;109:420-3.

8-Tytle TL, Johnson TH. Changing patterns in pulmonary tuberculosis. South Med J 1984; 77:1223-27.

9- Bock NN, McGowan JE Jr, Ahn J, Tapia J, Blumberg HM. Clinical predictors of tuberculosis as a guide for respiratory isolation policy. Am J Respir Crit Care Med 1996;154:1468-72.

10- Tattevin P, Egmann G, Casalino E, Fleury L, Ruel M, Bouvet E. The validity of medical history, classical syptoms, and chest radiographs in predicting pulmonary tuberculosis. Chest 1999; 115:1248-53.